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Please see attached in connection with our phone interview this afternoon regarding 08/484,340.

Methods in Enzymology

Volume 152

Guide to Molecular Cloning Techniques

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1987

ACADEMIC PRESS, INC.
Harcourt Brace Jovanovich, Publishers
Orlando San Diego New York Austin
Boston London Sydney Tokyo Toronto

[9] Nick Translation

By JUDY MEINKOTH and GEOFFREY M. WAHL

Nick translation, or more precisely nick translocation, is a specific procedure for incorporating radioactive nucleotides into double-stranded DNA. The method takes advantage of the ability of *Escherichia coli* DNA polymerase I to combine the sequential addition of nucleotide residues to the 3′-hydroxyl terminus of a nick [generated by pancreatic deoxyribonuclease (DNase) I] with the elimination of nucleotides from the adjacent 5′-phosphoryl terminus. Linear, supercoiled, nicked, or gapped circular double-stranded molecules can be labeled to specific activities $>10^8$ cpm/μg with deoxynucleotide 5′-[α-^{32}P]triphosphates by this technique.[1,2] Since the nicks are introduced at random sites in the duplex, the method generates a population of radioactive fragments which partially overlap each other. At saturating levels of nucleotide triphosphates the size of the fragments is determined by the DNase concentration. Fragments approximately 500–1500 nucleotides long produce optimal signal-to-noise ratios when hybridized to immobilized DNA or RNA (see this volume [45, 61]), presumably due to their ability to hybridize with each other in overlapping complementary regions to form "networks" or "hyperpolymers."[2] While experiments consistent with hyperpolymer formation of nick-translated probes have been reported,[3,4] the reproducibility and extent of hyperpolymer formation seem to be difficult to obtain, probably because of the critical dependence on probe size.[2,4] Longer probes have been correlated with higher backgrounds (G. Wahl, unpublished observations).

Nick Translation Reaction

1. Dilute stock DNase in 50 m*M* Tris–HCl at pH 7.4, 10 m*M* $MgCl_2$, 1 mg/ml DNase-free bovine serum albumin just before use. A DNase concentration of about 40–80 pg/μl is usually adequate.
2. Mix 2 μl 10× NT (nick translation) buffer [10× = 50 m*M* $MgCl_2$, 200–250 μ*M* each unlabeled deoxyribonucleoside triphosphate (dNTP), omitting those corresponding to the labeled nucleotide(s), 50 m*M* Tris–

[1] P. W. J. Rigby, M. Dieckmann, C. Rhodes, and P. Berg, *J. Mol. Biol.* **113**, 237 (1977).
[2] J. Meinkoth and G. Wahl, *Anal. Biochem.* **138**, 267 (1984).
[3] G. M. Wahl, M. Stern, and G. R. Stark, *Proc. Natl. Acad. Sci. U.S.A.* **76**, 3683 (1979).
[4] R. H. Singer, J. B. Lawrence, and C. Villnave, *BioTechniques* **4**, 230 (1986).

Nucleic acid hybridisation

a practical approach

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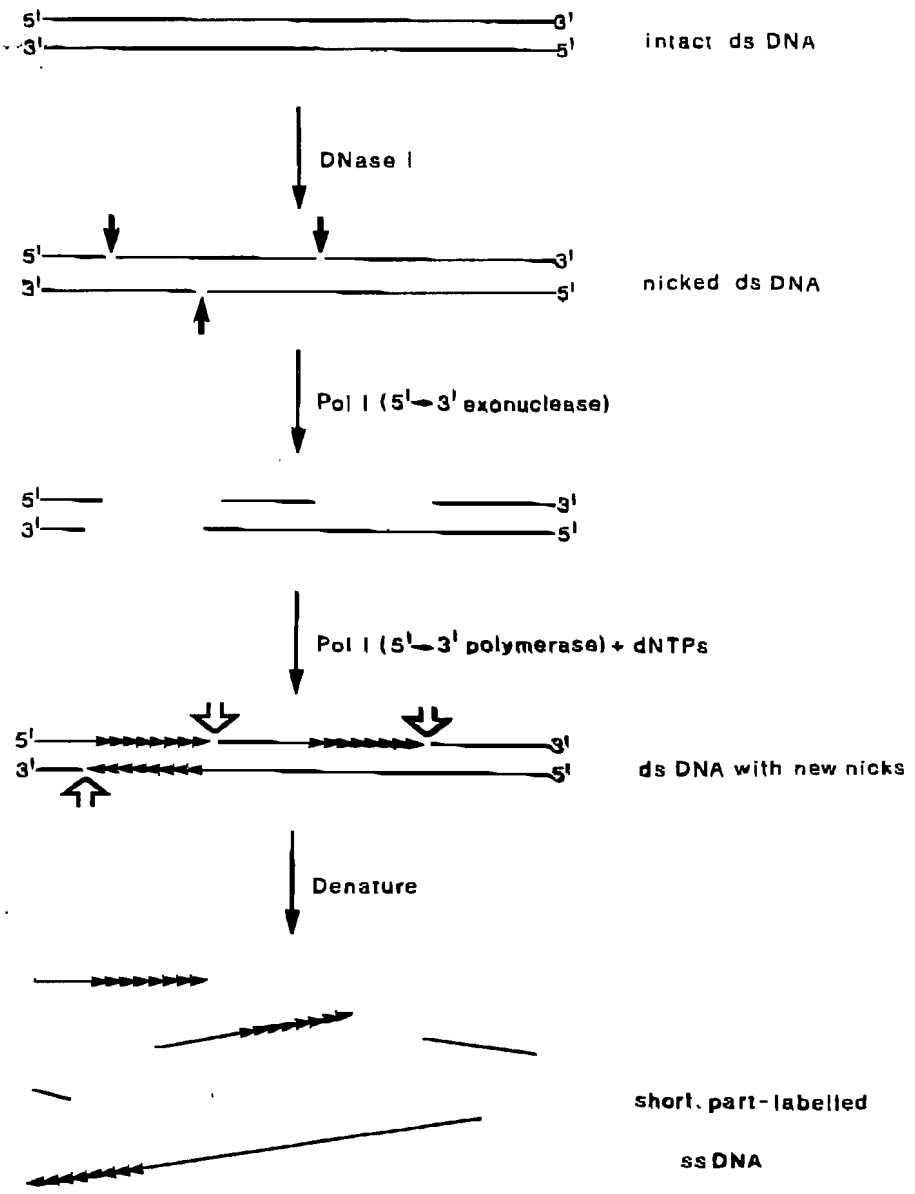

Figure 2. The preparation of probes by nick translation. ▼, original nick position; ▽, final nick position; ▬, labelled strand; Pol I, *E. coli* DNA polymerase I; dNTPs, deoxyribonucleoside triphosphates; ds and ss DNA, double- and single-stranded DNA, respectively.

Nick translation can utilise any deoxyribonucleotide labelled with ^{32}P in the α position. [^{125}I]-, [^{3}H]- and non-radioactive biotinylated nucleotides can also be incorporated. With α-^{32}P-labelled nucleotides, final specific activities of 5×10^8 d.p.m./μg DNA can be achieved. The detailed protocol is as follows.

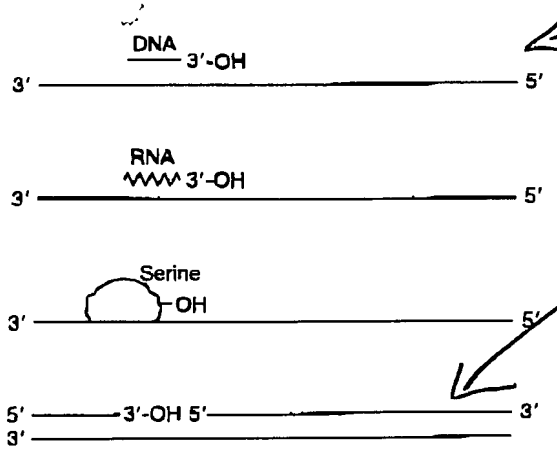

Figure 3.4 Four ways of generating 3'-OH goups necessary for DNA elongation, a deoxyribonucleotide oligomer, a ribonucleotide oligomer, a hydroxyl provided by a protein, and a nick.

molecules. Later one of the enzymes of DNA replication must remove the RNA primers.

After DNA polymerase III has elongated the lagging strand up to the next RNA primer, the enzyme releases from the DNA. Then DNA polymerase I binds and begins excising the ribonucleotides and at the same time adding deoxynucleotides at the 3' end (Fig. 3.5). As it moves, it translates the nick in the 5'-to-3' direction. The ability to substitute radioactive deoxyribonucleotides *in vitro* for nonradioactive ones by nick translation is a convenient method for making DNA radioactive for genetic engineering experiments. When the RNA primer has been completely removed and DNA pol I has released from the DNA, DNA ligase can bind and seal the

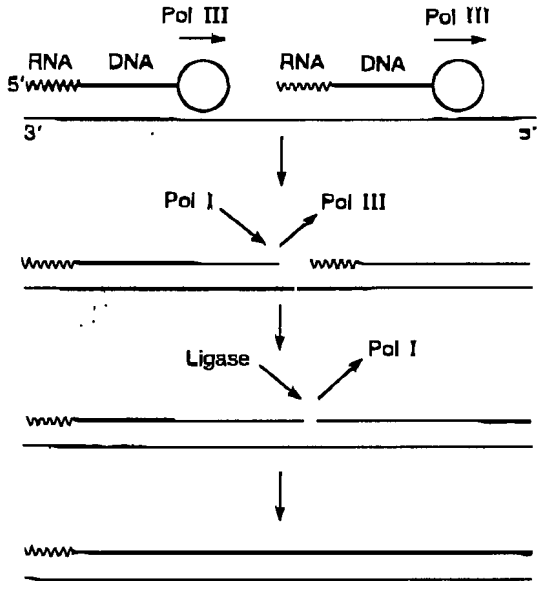

Figure 3.5 Conversion of elongating Okazaki fragments to a completed chain by the actions of DNA pol I and DNA ligase.

Genetics and Molecular Biology

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